Mem. Ins'. Butantan
24(2):85-100, Out.° 1952

A NEW METHOD OF QUANTITATIVE PAPER CHROMATOGRAPHY

BY K. SLOTTA AND J. PRIMOSIGH
(Research Department, Ind. Farm. Endochimica S/A)

The partition chromatography as indicated by Consden, Gordon and Martin (1), using filter paper as a support for the stationary phase, has since been applied by different authors for the quantitative determination of amino acids in artificial amino acid mixtures or protein hydrolysates respectively. The following principles have been applied:

1. Measuring of the area of the amino acids, colored with ninhydrin after an appropriate method of separation (2-5). The limit of error of this method is approximately ± 10%. Improvements on the original technique have been described (6, 7).
2. Elution of the amino acids after previous localization and determination of the concentration by means of appropriate micromethods (8-14). In this case it proved difficult to eliminate respectively to control the paper blank. By suitable pretreatment of the paper this method was improved and used successfully (with an error of ± 5%) for the determination of the amino acid composition of algal proteins (15).
3. Direct photometry of the amino acids, previously colored with ninhydrin on the paper (16-22). This method was applied for the determination of the amino acid concentration after twodimensional chromatography (18); prefractionation in groups and subsequent onedimensional chromatography (17-18); onedimensionally with buffered chromatograms (21) or onedimensionally with new kinds of solvent mixtures (low alcohols with additions (22): Under closely controlled conditions, particularly of the drying and coloring process, the probable error could be diminished to ± 4,36% (22). 4,36% (22).

Our experiments with crotoxin hydrolysates using a butanol-acetic acid mixture (23), gave sharp separations. Therefore the next step was to determine quantitatively the amino acids or amino acid groups separated in this manner.

Received for publication on October 30, 1952.

To determine the amino acids we chose the manometric ninhydrin carbon dioxide method of Van Slyke et al. (24). This excellent and very specific method is of great exactitude (25). In its submicro-and micro-execution it enables a measurement of 0.04 — 0.8 mg. of α-amino nitrogen with an error less than 1%. For this determination 10-15 mg. protein hydrolysate are necessary. This relatively large amount may be applied without overcharging the paper as a 2 cm. large streak on Whatman n.° 3 paper. To guarantee this we have constructed a simple apparatus which applies up to 1 ml. of solution very uniformly.

A typical chromatogram of this kind is reproduced in figure 1. In 144 hours the lactoglobulin hydrolysate, was separated into 7 distinctly defined zones. In the dripping off solution tyrosine, valine, methionine, phenylalanine and the leucines were present. Usually the paper was then dried for a short time at 90-95°, the amino acid zones localized by ultraviolet light, cut out, and every stripe eluted with O.1 N hydrochloric acid. After removing the hydrochloric acid by freeze-drying, the elution residue was dissolved in water and the amino acids determined in the Van Slyke-Neill-apparatus.

A large number of preliminary experiments were carried out to establish the separation capacity of Whatman n.° 3 paper for different amino acid concentrations, the adequate running time, time and temperature for drying as well as localization in the u. v. and elutibility of the different amino acids. Then artificial amino acid mixtures, containing the different amino acids in concentrations as might be expected in a lactoglobulin hydrolysate, were separated and determined.

TABLE I

Separation of an artificial amino acid mixture. Used: 1 ml. of an aqueous solution corresponding to 10 mg of β-lactoglobulin hydrolysate. pH = 6,0. Separation time: 144 hours. Solvent: butanol-acetic acid mixture.

	Exp. n.° 17	Exp. n.° 19	Exp. n.° 20
	recovered in %	recovered in %	recovered in %
	not determined	not determined	not determined
Cystine	101.9	97.4	97.5
Lysine	103.8	—	—
Histidine	100.4	96.2	96.0
Arginine	105.5	121.0	119.0
Aspartic acid + Glycine +	98.3	102.1	98.0
+ Serine	—	102.2	98.1
Glutamic acid + Threonine	99.0	100.4	99.4
	—	99.2	100.5
Alanine	97.1	103.2	97.0
Proline	Lost	97.3	97.0
		97.4	97.3

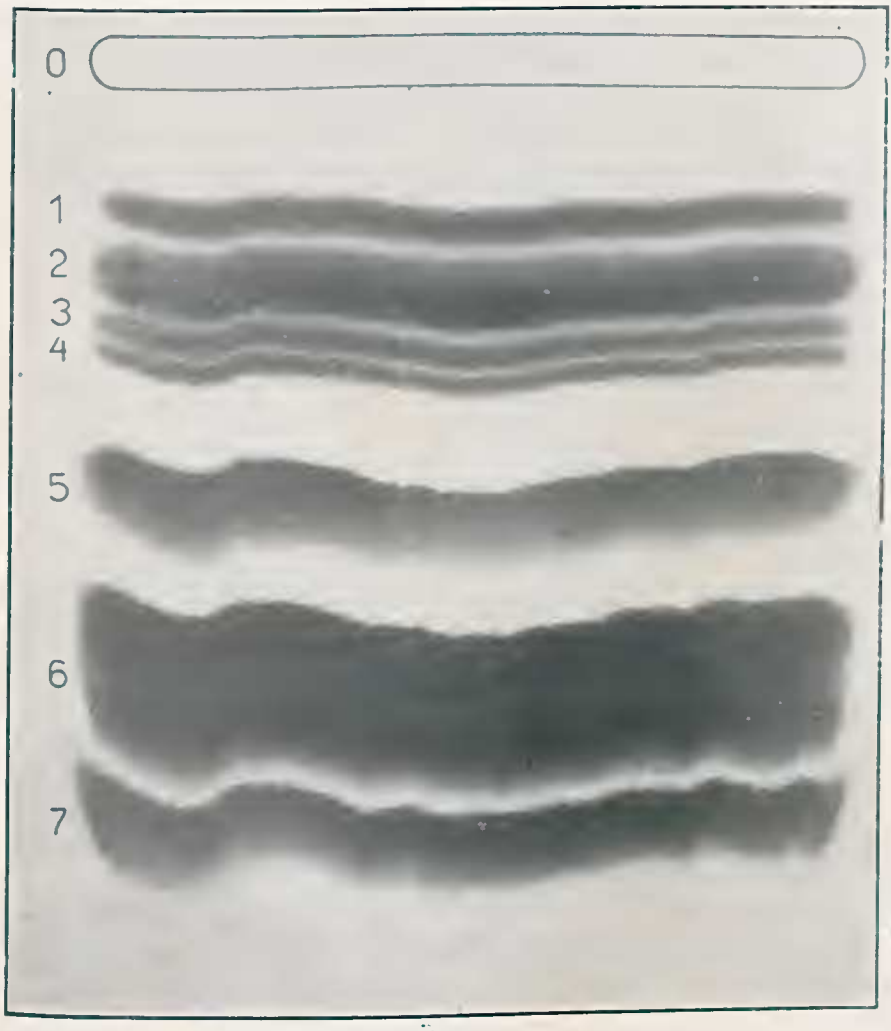


FIGURE 1

Separation of a β-Lactoglobulin hydrolysate on Whatman no 3 paper. 1 ml. hydrolysate containing 14.5 mg. of protein applied at "O". pH of the hydrolysate = 1.6. Separated by a mixture of butanol + acetic acid
Running-time 144 hours. Colored with ninhydrin. 1 = Cystine, 2 = lysine, 3 = histidine, 4 = arginine, 5 = aspartic acid + glycine + serine, 6 = glutamic acid + threonine, 7 = alanine + proline.

In these artificial mixtures cystine was not present: the pH of the solution was 6,0. At this pH the separation of arginine from aspartic acid is imperfect, the one of alanine from proline perfect. Later experiments with acid amino acid mixtures and protein hydrolysates of a pH = 1-2 showed that the pH of the applied solution has an influence on the separation. A low pH results in an excellent separation of arginine from aspartic acid; on the other hand the alanine proline separation is then imperfect. Subsequently we always used weakly acid hydrolysates and consider this procedure adequate, as the three bases can be determined very accurately in this manner. One may desist from the proline determination as this can be separated from alanine by other solvents (for example butanol-water).

As may be seen in table I the standard error is under ± 5% with the exception of arginine. The incomplete separation of arginine when using neutral solutions caused difficulties in the localization and cutting out of the arginine band. The slightest error in cutting resulted in arginine errors up to 50%, owing to the neighboring aspartic acid's relatively high concentration. But as may be seen in figure 1 this source of error could be avoided by using weakly acid hydrolysates, thereby enlarging the free intermediary zone.

Tables II and III show the results achieved by the above described method of separating and measuring the hydrolysates of two purified and crystallized proteins: crystallized β-lactoglobulin and crystallized lysozyme. The values for tyrosine, valine + methionine, phenylalanine and leucine + isoleucine given in lines 8-11 should be considered as preliminary. They were obtained by a 48 hour chromatography of the hydrolysates with the butanol-acetic acid mixture; under these conditions the said amino acids did not migrate from the paper. But the separation is not perfect and streaks of artefacts make localization difficult. This is why we consider the values as less accurate than those of the amino acids and amino acid groups in the lines 1-7. A perfect separation of the amino acids, dripping off under our standard conditions, will only be possible by using other solvent mixtures (benzylalcohol, butanol-water).

Although the analysis of β-lactoglobulin is incomplete, a short discussion of the results seems advisable. The cystine values were 20-25% lower than those obtained by Brand (*26*) under adequate conditions of hydrolysis. The cystine value also showed larger oscillations among themselves (see lysozyme); particularly prolonged heating of the paper led to larger losses in the case of cystine. We have not yet investigated whether cystine may be quantitatively determined by this method under adequate conditions of hydrolysis.

Our lysine value is a little higher than that of Brand (26) and of Lewis (28), but not as high as Stein and Moore's (*27*). In spite of the low concentration, histidine could be determined exactly. The arginine value was app. 6 % too low.

TABLE II

Amino acid composition of β-lactoglobulin. Results in gm. of α-amino nitrogen per 100 gm. of protein.

	Paper chrom. 1	Paper chrom. 2	Brand et al. (26)	Stein & Moore (27)	Lewis et al. (28)
Cystine	0,306	0,309	0,396	—	—
Lysine *	1,111	1,146	1,093	1,205	1,074
Histidine	0,149	0,153	0,143	0,147	0,150
Arginine	0,221	0,219	0,232	0,234	0,232
Aspartic acid || + Glycine // + Serine **	3,061	3,053	3,236	3,130	3,067
Glutamic acid + Threonine *** ...	2,242	2,249	2,544	2,366	2,363
Alanine + Proline §	1,711	1,741	1,479	1,746	1,526
Tyrosine	0,339	—	0,292	0,281	0,299
Valine + Methionine	0,988	—	1,000	0,974	1,034
Phenylalanine	0,288	—	0,300	0,321	0,308
Leucine + Isoleucine	2,301	—	2,563	2,281	2,403

* The experimentally obtained lysine values were divided by 1,10.

|| The double value of aspartic acid-N was used in calculating the group-nitrogen of the reference protein.

// 90 % of the glycine-N value were used in calculating group-N of the reference protein.

** Uncorrected serine values were used in the calculation of the group-N of the reference protein.

*** Uncorrected threonine values were used in the calculation of the group-N of the reference protein.

§ 101 % of the proline value were used in the calculation of the group-N of the reference protein.

The value for the group aspartic acid + glycine + serine is nearly identical with the one of Lewis and in good accordance with Stein and Moore's. The difference between our values and those of Brand is caused by his obviously too high serine value. The value for glutamic acid + threonine coincides within 5% with the one of Stein and Moore as well as with Lewis'. The far higher value of Brand can be explained by his high value for threonine. Alanine and proline are amino acids which formerly could not be accurately determined by microbiological methods. The proline value of Brand, obtained by microbiological methods, could not be confirmed by Lewis who also applied microbiological determinations. It is really higher, and although the alanine values of both authors are fairly equal, Lewis considers his alanine value as uncertain, presumably 10-20 % too low. Our value for alanine + proline confirms the supposition that both values of Brand are too low and that the alanine value of Lewis is also too low. Our values agreed well with the one of Stein and Moore, but we found a much higher value than Brand and a higher one than Lewis.

We consider the tyrosine value as uncertain, as it was impossible to localize tyrosine beyond doubt and cut it out neatly. The valine + methionine group was in good agreement with the three comparative values and phenylalanine is in accordance within 5 % with Brand's value. The value for the leucine + isoleucine group corresponds very closely to the one found by Stein and Moore. The higher value of Brand et al. is caused by the high isoleucine value obtained by microbiological methods; it has since been corrected (29) and now corresponds, more or less, to the one found by Stein and Moore through partition chromatography on a starch column.

TABLE III.

Amino acid composition of lysozyme. Results in gm. α-amino nitrogen per 100 gm. of protein.

	Paper chromat.		*Fromageot et al.* (30)	Lewis et al. (28)
	1	2		
Cystine	0,775	0,668	0,933	—
Lysine *	0,524	0,555	0,578 *)	0,546
Histidine	0,166	0,091	0,105 *)	0,094
Arginine	0,999	1,087	1,054 *)	1,022
Aspartic acid || + Glycine // + Serine **	5,714	5,761	5,298	5,594
Glutamic acid + Threonine ***	0,980	1,022	0,913	1,058
Alanine + Proline §	1,391	1,437	1,119	1,084
Tyrosine	0,402	—	0,286	0,277
Valine + Methionine	0,756	—	0,778	0,768
Phenylalanine	0,283	—	0,195	0,265
Leucine + Isoleucine	1,307	—	1,463	1,292

*) Values from a recently published paper were used here (31).

* The experimentally found lysine values were divided by 1,10.

|| The double value of aspartic acid-N was used in the calculation of the group-nitrogen of the reference protein.

// 90 % of the glycine-N value were used in calculating the group-N of the reference protein.

** Uncorrected serine values were used in the calculation of the group-N of the reference protein.

*** Uncorrected threonine values were used in calculating the group-N of the reference protein.

§ 10: % of the proline value was used in the calculation of the group-N of the reference protein.

Lysozyme is a protein with an uncommonly high tryptophane content and gives strongly colored hydrolysates. The analysis was made difficult by streaks of artefacts which appeared in the neighbourhood of arginine, proline and tyrosine. A very narrow band was once observed between aspartic and glutamic acid.

What has been said in the discussion of the lactoglobulin value goes for cystine too. The oscillation of the lysine values is larger than usual; the mean

value corresponds to the one of Lewis. Lysozyme contains histidine in a very low concentration and gave an absurd value for this constituent in experiment no. 1. On account of a streak of artefact the localization of histidine proved very difficult and apparently part of the arginine was found with the histidine. In experiment no. 2 the value coincides within 5 % with the one found by Lewis, but this must be considered as rather a chance happening, as at this low concentration the limit of this methods accuracy has been reached. Arginine was found somewhat higher in experiment no. 2, but coincides within 5 % with the value found by Fromageot. The group aspartic acid + glycine + serine presented much higher values than those found by Fromageot and they were also about 3% above those of Lewis. Probably the low values of Fromageot were caused by his too low aspartic acid value. Our average value of glutamic acid + serine is midway between the values of Fromageot and Lewis. Lewis finds a value of 4,32 % for glutamic acid and calculates 4,36 residues per molecule therefrom. Fromageot calculates a value of 3,3 residues per molecule from a concentration of 3,3 %. Most probably our value of 4 residues per molecule is right. It is as yet not quite certain what caused our relatively high value for the alanine + proline group. The average value from the two experiments is 26 % higher than Fromageot's and 30 % higher than Lewis'. Although Lewis refers to his alanine value obtained by microbiological methods as probably 10-20 % too low, this does not explain the increase of 30 % in our value. Probably the reason for this increase is a double one: 1) an actually higher alanine value and 2) a slight increase of the value found on account of the artefact directly adjacent to proline.

The value for tyrosine cannot be used in the case of lysozyme because cutting out was rendered most difficult by incomplete separation, even more than with β-lactoglobulin. The group valine + methionine coincides with both comparative values within 5 %. Our values for phenylalanine and the leucine-isoleuleucine group confirm those of Lewis; the phenylalanine value of Fromageot is definitely too low. The increase of the group value leucine + isoleucine of Fromageot is caused by his higher value for isoleucine.

From this detailed discussion of the results it may be seen how much remains to be done till this method can be transformed into one for a reliable and complete analysis of protein hydrolysates.

From the groups aspartic acid + glycine + serine and glutamic acid + threonine the two dicarboxylic acids may be separated without difficulty by means of an anion exchanger and then determined as individual amino acids. Preliminary experiments in this direction gave good results.

Another possibility arises from the use of different solvent mixtures (22), resp. of buffered solvent mixtures (32). As every amino acid can be obtained separately in the onedimensional chromatograms, the chief problem nowadays

consists in applying the most rational solvent mixture. It should separate the largest number of amino acids in a precise and easily reproductible way. Experiments in this direction have been carried out.

We think it is too early to compare the exactitude of this method with other ones of longer standing and experience. On the one hand, this method will allow only the determination of a few individual amino acids, while on the other the small paper blank could not yet been eliminated and artefacts are present which render the cutting out difficult. From our experiments with artificial amino acid mixtures, which can be separated without overlapping, we may deduce that an exactitude of ± 2 % can easily be achieved in those cases where a good separation and a sufficiently high concentration 0.3-0,5 mg. of NH_2-N) make an accurate determination possible. The still existing small paper blank could probably be completely eliminated by the very efficient purifying procedure described by Hanes et al. (33).

EXPERIMENTAL PART

1. *Reagents used.*

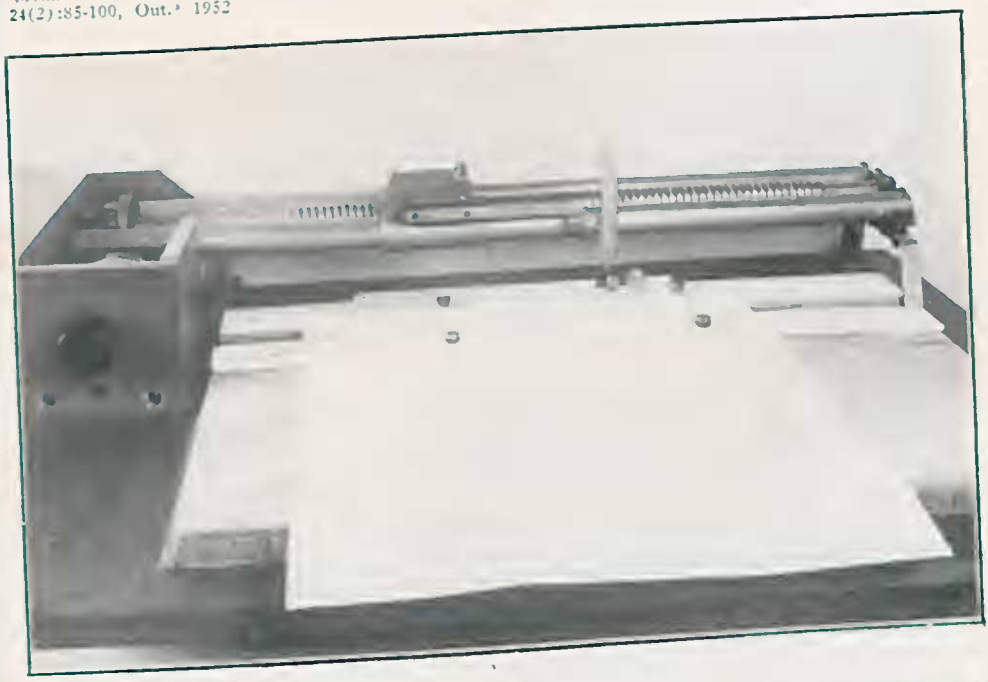
With the exception of isoleucine from the Nutritional Biochemicals Corp., Cleveland, Ohio, all the amino acids used were products of the H. M. Chemical Co., Los Angeles, California. All the amino acids were C. P. and when dried gave theoretical Kjeldahl values within ± 0,5 %, with the exception of L-arginine which contained sodium chloride. In spite of the satisfactory value of total nitrogen L-proline contained oxyproline which appeared in the paper chromatogram. Oxyproline could be determined semiquantitatively and amounted to about 2 % of the sample.

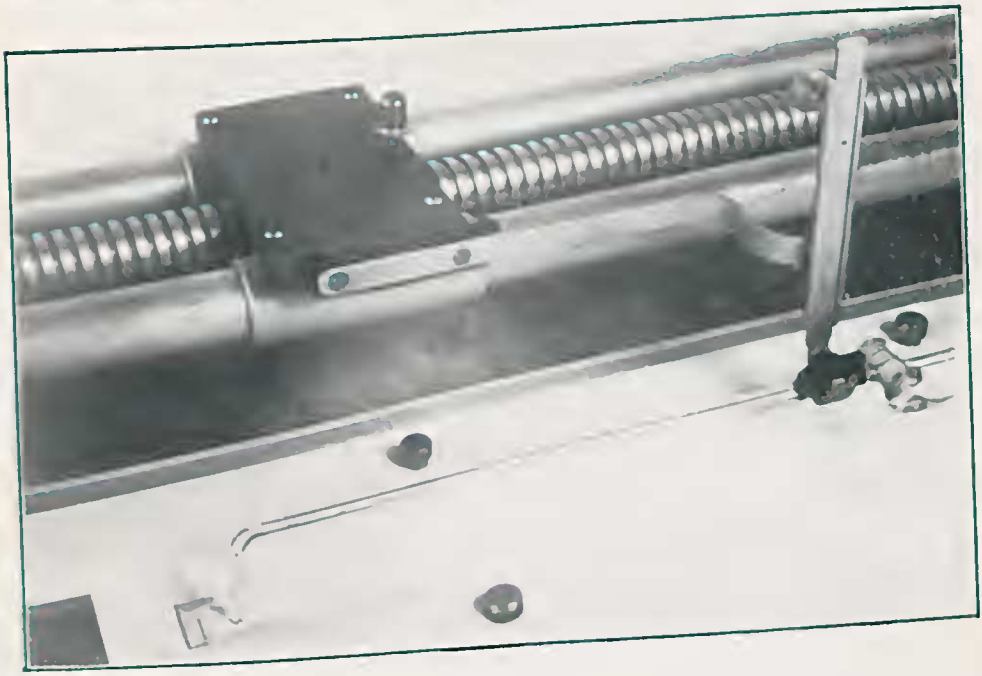
Crystallized β-lactoglobulin and crystallized lysozyme were products of the Armour Laboratories, Chicago, Ill. They were used without further crystallization. We calculated on the basis of a total nitrogen value of 15,6 % (26, 27) for lactoglobulin, resp. 18,6 % (30) for lysozyme.

The mixture used for separation consisted of butanol + glacial acetic acid + water in the proportion of 4:1:5 (v/v). All solvents were p. a. preparations.

2. *Hydrolysis.*

Each time 400 mg. of air dried protein were hydrolysed for 18 hours with 50 ml. of 6 N hydrochloric acid in an electrically heated glycerol bath under reflux. The temperature of the bath was adjusted to 125.° C. The hydrolysate of lactoglobulin had a light yellow color and that of lysozyme a very dark brown. The principal amount of hydrochloric acid was distilled in the usual manner in the vacuum; the syruplike residue was dissolved in water and frozen





FIGURES 2 and 3

Applying apparatus with pipette

in a freezing mixture. The hydrolysate was left in high vacuum with dry silicagel and soda lime for the absorption of the hydrochloric acid and was completely dry after app. 60 hours. The residue was dissolved in warm water, transferred quantitatively into a 25 ml. volumetric flask and completed with water. Samples of 1 ml. each of the hydrolysate diluted in this manner, containing 10-15 mg. of protein, were used to determine the total nitrogen and also for the paper chromatogram. The pH of this solution was 1,4-1,6. At this pH the separation of the bases is perfect, and care must be taken that the pH of the applied solution is 1-2.

3. *Applying the sample.*

All quantitative experiments were carried out with untreated Whatman no. 3 paper (46 x 57 cm.). In the first experiment the sample was applied 10 cm. from the upper rim of the paper with a micro-burette in 24 portions of 0,025 ml. each. The total volume amounted therefore to 0,6 ml. The streak had a length of app. 38 cm. and contained 6-9 mg. of dissolved substance. If the application is done evenly, relatively undistorted stripes may be obtained after the separation. But it is preferable to use an apparatus which makes very regular application possible and has the further advantage of distributing up to 1 ml. of hydrolysate evenly, while at the same time keeping the length of the streak unaltered.

Following a suggestion of Yanovsky et al. (34) we used at first a modified kymograph on which we clamped the paper sheet. As difficulties arose concerning the exact dosage of the sample to be applied we chose another procedure: the solution is applied to the stationary paper with a pipette which is drawn over the sheet at an even speed. Figure 2 is a photograph of the apparatus and pipette at the beginning of the run. The movement of a motor is transmitted to an endless screw, which moves a carriage with the pipette. The paper is stretched over a board and hold at the desired position by means of two steel bars wrapped in filter paper. Between these rails there is a groove in the board, 5 cm. wide and 0,5 cm. deep; this prevents the humid paper from coming into contact with the wooden board.

A measuring pipette (Kimble Exax) of 1 ml., graduated in 0,01 ml., was used. Its tip end was bent at a right angle and the suction end provided with a stopcock. The tip was first closed and drawn out a little on a blast burner and than opened again by careful grinding so that the size of the opening was adequate to apply a volume of 1 ml. as a streak 38-39 cm. long at pipette speed of 0,45 cm./ sec. On account of the fine opening of the pipette it was necessary to use a vacuum pump to fill it. Then the stopcock was closed and the pipette hooked into the vertically movable metal support of the carriage. The tip was placed on a piece of filter paper (starting paper) which was protected under-

neath by impermeable paper. The carriage was put into motion, the stopcock opened and the liquid began to flow out onto the starting paper. As soon as the zero point of the pipette was reached, the starting paper was pulled away with a quick movement and then the pipette slid on the paper sheet. As soon as the desired volume had been applied, the pipette was lifted and the stopcock closed. The pipette should not start applying less than 3 cm. from the lateral rim of the paper, because in the course of the 6 days of chromatography the streaks grow longer and they should never be allowed to extend to the rim of the paper.

4. *Chromatography.*

The loaded paper is left to dry for 15 minutes at room temperature and then completely dried at 60-70.° C, preferably by means of a hair dryer.

It is advisable to stipulate the running time by the amount of solvent used for the separation. As a rule we introduced two paper sheets in the trough as described by Consden et al. (1), and filled it with 250 ml. of the butanol-acetic acid mixture. After two days it was possible to add the remaining 160 ml. We took the papers out of the chamber after all the liquid (410 ml. for two sheets) in the container had been used up. This lasted 6-7 days. 200 ml. butanol-acetic acid mixture for two sheets of paper were used for the preliminary separation, which helps to determine the amino acids normally passing into the extract. This procedure of separation was finished in app. 60 hours.

5. *Marking and eluting.*

Thereafter the sheets and the trough are removed from the chamber and dried first in a cold stream of air, then for 20 minutes at 90-95.° C to make the stripes visible under u. v. When using acid hydrolysates (pH = 1-2) we could observe stripes of artefacts originating from the hydrolysate (tryptophane decomposition products, particularly marked in lysozyme hydrolysates) as well as others originating from the paper. It is therefore indispensable to run a second sheet in each experiment which is afterwards colored with ninhydrin. This serves to ascertain the localization in the u. v. of the amino acids on the other sheet. The places marked with pencil are cut out resulting in 7 paper stripes of different width, which are eluted in an elution chamber of adequate size by means of 0, 1 N hydrochloric acid following the method described by Dent (35), whereby the amino acids in the stripe of filter paper, clamped between two slides, are eluted quantitatively by the acid. The bottom end of the paper stripe was cut to a point, so as to guarantee better dripping off.

The first elution experiments were carried out with water. Although we succeeded in eluting the neutral amino acids quantitatively, arginine was only eluted up to 80 %, lysine up to 85 % and the dicarboxylic acids up to 90 %.

The use of 0,1 N hydrochloric acid has two advantages: 1) complete elution of all the amino acids and 2) reduction of the paper blank to a low and constant level by decomposition of the carbonates eluted from the paper. We proceded with the elution in a manner to wash out all the paper stripes for 16 hours, independent of their width. The extract of the narrow stripes amounted to app. 5 ml. and that of the widest (glutamic acid + threonine) to app. 25 ml.

The glass tubes used to receive the extract and for the following freeze drying were of 2,7 cm. diameter, 11,5 cm. height and having a constriction 4 cm. under the rim. This prevents the escape of the ice-plug, in case small quantities of liquid start boiling on the bottom of the tube under high vacuum, thereby expelling the ice-plug. The capacity up to the constriction was about 35 ml.

The frozen samples were brought into exsiccators half filled with dry silicagel and a small beaker of soda lime. A vacuum, better than 1 mm., was produced by means of a small oil pump. In 24-48 hours the samples were dry as dust and were left in the exsiccator up to the time of re-dissolution. Leaving them outside the exsiccator resulted in absorption of humidity from the air and liquefaction of the samples; whereby the volume of the residue is increased, which has o be avoided, because when dissolving the sample in 4,4 resp. 2,4 ml. the volume of the dry residue is insignificant and need not be taken into consideration.

6. Determination.

We used the Van Slyke-Neill portable manometric gas analysis apparatus furnished by A. H. Thomas Co., Philadelphia. The all glass reaction vessels (36) were convenient and absolutely air tight when greasing the stopcocks with a mixture of 5 parts petrolatum and 1 part of crude rubber (37). With the exception of the two-way stopcock of the extraction chamber, which was greased with Silicone high vacuum grease, all the other stopcocks were treated with petrolatum crude rubber mixtures. We used 0,5 N sodium hydroxide saturated with sodium chloride and 2 N lactic acid. The hydrazine sulfate (25) was dissolved in the 2 N lactic acid (38). The calculation factors for the COOH-N were taken from Mac Fadyen's publication (39).

The routine procedure for the determination was the following: — The sample, contained in the all glass reaction vessel in a volume of 2 ml., was left with 100 mg. solid ninhydrin and 100 mg. solid citrate buffer of a pH = 2,5 in a boiling waterbath for 8 minutes. Several blank tests with the same amount of ninhydrin and citrate buffer in 2 ml. ran parallel and gave 6-7 mm. mercury measured at the 2,0 ml. mark and 25-30 mm. mercury measured at the 0,5 ml. mark (24).

The abnormally reacting amino acids gave the values shown in table IV; therefore the experimentally found value for lysine was divided by 1,10, the one for proline by 1,01 and the one for glycine by 0,90.

TABLE IV

Carbon dioxide development of some abnormally reacting amino acids. Volume = 2 ml., pH = 2,5. Boiled with ninhydrin for 8 minutes.

	Our values % COOH-N	Van Slyke et al. (24) % COOH-N	Schott et al. (25) % COOH-N
Lysine	110	105	109
Proline	101	100	102
Glycine	89.5	95	91-92

Cystine could be determined best at pH = 1 (0,1 N hydrochloric acid without addition of buffer) and after boiling it with ninhydrin for 5 minutes, gave theoretical values.

As a rule we dissolved the dry elution residue in 4,4 ml. of water in the lyophilizing tubes without taking its volume into consideration. This dilution was preferred for the stripes which were expected to have a higher amino acid concentration, because it made two recordings for each sample possible. But the histidine, tyrosine and phenylalanine residues were dissolved in only 2,4 ml., as the expected concentration was very low and the procedure did not allow a control determination. Cystine was dissolved in 0,1 N hydrochloric acid and determined without adding any buffer.

In spite of the high specificity of the manometric ninhydrin carbon dioxide method we did not succeed in the total elimination of the paper blank. It seems to be brought about by a substance with the qualities of a higher peptid (33, 40). Possibly it can be removed by an adequate preliminary treatment of the paper (40), which we have not yet tried; therefore it was necessary to do a separate determination of the small blank value of the paper. For this purpose it is best to run a sheet of paper parallel under identical conditions, then cut it up in stripes of the same width as those containing the different amino acids and finally elute them with 0,1 N hydrochloric acid. The blank values are very small and constant and do not amount to more than 1 mm. mercury for wide paper stripes and 0,2 mm. for narrow ones, determined at the 2 ml. mark., resp. 3-5 mm. mercury for wide stripes and 2-3 mm. for narrow stripes, measured at the 0,5ml. mark. If the paper blank is not taken into consideration. plus errors up to 5 % may occur.

RESUMO

Um método de determinação quantitativa dos amino ácidos em hidrolisados protéicos por meio de cromatografia de partição foi descrito.

10-15 mg de um hidrolisado protéico são aplicados em forma de banda sôbre papel Whatman n.º 3 e, após a separação, cada constituinte foi determinado pelo método manométrico de Van Slyke-Neill (ninhidrina-anidrido carbônico). Desta maneira a lisina, a histidina, a arginina, a tirosina e a fenilalanina são determináveis como amino ácidos individuais, enquanto que o ácido aspartico + glicina + serina, o ácido glutâmico + treonina, a alanina + prolina, a valina + metionina e a leucina + isoleucina são determináveis como grupos.

O método foi aplicado para a determinação dos amino ácidos numa mistura modêlo, e o erro da determinação era inferior a ± 5 %.

Uma análise de duas proteinas cristalisadas, a saber, a β-lactoglobulina e a lisozima, deu valores em perfeito acôrdo com aqueles descritos na literatura.

RESUMÉ

Une méthode de détermination quantitative des acides aminés dans des hydrolysats protéiques à l'aide de chromatographie de partition a été décrite.

10-15 mg d'un hydrolysat protéique ont été appliqué sous forme de bande sur papier Whatman No. 3 et après la séparation, chaque constituant a été déterminé par la méthode manométrique ninhydrine-anhydride carbonique de Van Slyke-Neill. De cette manière, la lysine, la histidine, l'arginine, la tyrosine et la phenylalanine sont déterminables comme des acides aminés individuels, tandis que l'acide aspartique + glycine + serine, l'acide glutamique + thréonine, l'alanine + proline, la valine + méthionine et la leucine + isoleucine, comme groupes.

La méthode a été appliquée pour déterminer les acides aminés dans un mélange modèle, et l'erreur de détermination fût inférieure à ± 5%.

Une analyse de deux protéines cristallisées, à savoir, la β-lactoglobuline et le lysozyme, a donné des valeurs en parfait accord avec celles décrites dans la littérature.

ZUSAMMENFASSUNG

Es wird eine Methode zur quantitativen Bestimmung von Aminosäuren in Eiweisshydrolysaten mittels Verteilungschromatographie beschrieben. Bei Verwendung von Whatman No. 3 Papier werden 10-15 mg Proteinhydrolysat streifenförmig aufgetragen und nach der Trennung die einzelnen Komponenten mit

der manometrischen Ninhydrin-Kohlendioxyd-Methode von Van Slyke-Neill bestimmt. Auf diese Weise sind Lysin, Histidin, Arginin, Tyrosin und Phenylalanin als individuelle Aminosäuren, und Asparaginsäure + Glycin + Serin, Glutaminsäure + Threonin, Alanin + Prolin, Valin + Methionin und Leuzin + Isoleuzin als Gruppen bestimmbar.

Die Methode wurde zur Bestimmung von Aminosäuren in Modellgemischen verwendet und der Fehler der Bestimmung lag unter ± 5 %.

Die Analyse zweier krystallisierter Proteine, des β-Laktoglobulins und des Lysosyms ergab Werte, die mit den in der Literatur beschriebenen übereinstimmten.

BIBLIOGRAPHY

1. Consden, R., Gordon, A. H. and Martin, A. J. P. — *Biochem. J.* 38: 224, 1944.
2. Polson, A., Mosley, V. M. and Myckoff, R. W. G. *Science* 105: 603, 1947.
3. Polson, A. — *Biochim. Biophys. Acta* 2: 575, 1948.
4. Fisher, R. B., Parsons, D. S., and Morrison, G. A. *Noture* 161: 764, 1948.
5. Berry, H. K. and Cain, T. — *Arch. Biochem.* 24: 179, 1949.
6. Fromageot, C. and Privat de Garilhe, M. — *Biochim. Biophys. Acta* 4: 509, 1949.
7. Vianna, R. C. and Diniz, C. R. — *Ciência e Cultura* 3: 38, 1951.
8. Woiwod, A. J. — *Nature* 161: 169, 1948.
9. Naftalin, L. — *Nature* 161: 763, 1948.
10. Awapara, J. — *J. Biol. Chem.* 178: 113, 1949.
11. Landua, A. J. and Awapara, J. — *Science* 109: 385, 1949.
12. Martin, A. J. P. and Mittelmann, R. — *Biochem. J.* 43: 353, 1948.
13. Boissonas, R. A. — *Helv. Chim. Acta* 33: 1975, 1950.
14. Fowden, L. — *Biochem. J.* 48: 327, 1951.
15. Fowden, L. — *Biochem. J.* 50: 355, 1952.
16. Rockland, L. B. and Dunn, M. S. — *J. Am. Chem. Soc.* 71: 4121, 1949.
17. Block, R. J. — *Science* 168: 608, 1948.
18. Block, R. J. — *Anol. Chem.* 22: 1327, 1951.
19. Bull, H. B., Hahn, J. W. and Baptist, V. H. — *J. Am. Chem. Soc.* 71: 551, 1949.
20. Fosdick, L. S. and Blackwell, R. Q. — *Science* 109: 314, 1949.
21. McFarren, E. F. and Mills, J. A. — *Anol. Chem.* 24: 650, 1952.
22. Redfield, R. and Barron, E. S. G. — *Arch. Biochem. Biophys.* 35: 443, 1952.
23. Slotta, K. and Primosigh, J. — *Mem. Inst. Butantan* 23: 51, 1951.
24. Van Slyke, D. D., Dillon, R. J., Mac Fadyen, D. A. and Hamilton, P. B. — J. *Biol. Chem.* 141: 627, 1941.
25. Schott, H. F., Rockland, L. B. and Dunn, M. S. — *J. Biol. Chem.* 154: 397, 1944.
26. Brand, E., Saidel, L. J., Goldwater, W. H., Kassel, B. and Ryan, F. J. — *J. Am. Chem. Soc.* 67: 1524, 1945.
27. Stein, W. H. and Moore, S. — *J. Biol. Chem.* 178: 79, 1949.
28. Lewis, J. C., Snell, N. S., Hirschman, D. J. and Fraenkel-Conrat, H. — *J. Biol. Chem.* 186: 23, 1950.

29. Smith, E. L. and Greene, R. D. — *J. Biol. Chem.* **172**: 111, 1948.
30. Acher, R., Jutisz, M. and Fromageot, C. — *Biochim. Biophys. Acta* **5**: 493, 1950.
31. Monier, R., Gendron, Y., Jutisz, M. and Fromageot, C. Biochim. Biophys. Acta **8**: 588, 1952.
32. McFarren, E. F. — *Anal. Chem.* **23**: 168, 1951.
33. Hanes, C. S., Hird, F. J. R. and Isherwood, F. A. *Biochem. J.* **51**: 25, 1952.
34. Yanofsky, Ch., Wasserman, E. and Bonner, D. M. *Science*, **111**: 61, 1950.
35. Dent, C. E., Stepka, W. and Stewart, F. C. — *Nature* **160**: 682, 1947.
36. Hamilton, P. B. and Van Slyke, D. D. — *J. Biol. Chem.* **150**: 231, 1943.
37. Peters, J. P. and Van Slyke, D. D. — Quantitative Clinical Chemistry, Vol. II. Baltimore 1932.
38. Hamilton, P. B. and Van Slyke, D. D. — *J. Biol. Chem.* **164**: 249, 1946.
39. Mac Fadyen, D. A. — *J. Biol. Chem.* **145**: 387, 1942.
40. Wynn, V. — *Nature* **164**: 445, 1949.